EDITORIAL

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Antibiotics v2.0: computational and synthetic biology approaches to combat antibiotic resistance

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“...only two new classes of antibiotics have reached the market since the 1960's.”

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Antibiotic-resistant pathogens cause significant morbidities and mortalities each year, killing approximately 700,000 people globally; a figure that is predicted to increase to 10 million by 2050 [1]. Treatment of bacterial infections is increasingly hampered by rapidly growing rates of antibiotic resistance, which is exacerbated by the combined overuse and misuse of antibiotics in medicine and agriculture. A recent declaration following a meeting of the UN General Assembly stated, “antibiotic resistance is the greatest and most urgent global risk, requiring increased attention and coherence at the international, national and regional levels” [2]. There, thus, exists an urgent need for new antimicrobials; an imperative reinforced by the fact that only two new classes of antibiotics have reached the market since the 1960's [3]. Recent reports of bacteria isolated from animals and humans which are resistant to the so-called last-line antibiotic, colistin, is a worrying development, especially given that the resistance determinant, *mcr-1*, is plasmid-encoded and thus mobile [4,5]. In the last year alone,

numerous reports have emerged confirming the spread of *mcr-1* globally [6], and in some cases, in combination with extended-spectrum β -lactamase production [7]. Colistin resistance is not a new phenomenon, but the fact that the *mcr-1* gene is horizontally transmissible, highlights a continuously evolving landscape in antimicrobial resistance. This is in addition to the identification of novel antimicrobial resistance genes and atypical mechanisms of resistance, for example, a novel tetracycline efflux system from the oral metagenome [8] and intracellular antibiotic inactivation [9]. Even more worryingly, a carbapenem-resistant Enterobacteriaceae *Klebsiella pneumoniae* isolate resistant to all available antibiotics in the USA was linked to a fatal infection in a patient who had recently returned from India [10]. The strain displayed pan-resistance to 26 antibiotics, including all aminoglycosides and polymyxins, yet was *mcr-1*-negative. Taken together, these reports highlight the hastening slide toward the emergence of pan-resistant microbial infections.

KEYWORDS

- antibiotic resistance • antimicrobial
- bacteriocin • bioinformatics
- biosynthetic gene cluster • colistin
- *mcr-1* • multi-drug resistance
- synthetic biology • synthetic DNA

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Proposals on coping with the crisis, such as the comprehensive ‘O’Neill Report’ on antimicrobial resistance [1], details specific intervention strategies and states that, “many known avenues of research for AMR have failed; we need to go back to the drawing board and look for new ones.” In line with this call to arms, one promising approach is the use of bioinformatics in combination with synthetic biology and DNA and peptide synthesis to identify and produce novel antimicrobials, by mining genome and metagenome sequence data for biosynthetic gene clusters (BGCs).

BGCs encode antimicrobial compounds, such as polyketide antibiotics, nonribosomal peptides, alkaloids, saccharides, terpenoids and bacteriocins (ribosomally synthesized and post-translationally modified peptides) [11] which have the potential to control pathogenic microorganisms [12]. Approximately 70% of antimicrobials currently used in medicine are products or derivatives of bacterial and fungal secondary metabolism. The vast amounts of genome and metagenome sequence data generated provide an almost limitless supply of genes to be ‘mined’ for novel antimicrobials. A large proportion of such microorganisms are yet to be cultured in the laboratory, but crucially, advances in DNA sequencing and synthesis have enabled researchers to access the enormous diversity of antimicrobials produced, providing a means to study these organisms without the need for laboratory culture.

For example, a recent study identified more than 14,000 BGCs in the human microbiome, of which a subset of approximately 3000 were examined in more detail [13], while dozens of bacteriocin gene clusters were identified by analyzing the Human Microbiome Project gut metagenome dataset [14]. Numerous bioinformatic databases and algorithms exist to identify such BGCs (detailed in Medema and Fischbach [15]). Encouragingly, a number of studies have begun to take this approach a step further than high-throughput bioinformatic screening and actually synthesize antimicrobials (named synthetic-bioinformatic natural products) based on primary sequence data alone. Focusing on nonribosomal peptide synthetases and taking advantage of advanced algorithms for predicting the chemical structure from sequence data, allowed Chu *et al.* to synthesize novel antimicrobials (humimycins) which were active against methicillin-resistant *Staphylococcus aureus* clinical isolates and which enhanced the effect of certain β -lactam

antibiotics and increased murine survival following infection [16]. This is a powerful approach, which holds significant future promise as it overcomes: the need to culture the producing organism, the fact that many BGCs remain silent under laboratory conditions and gene expression issues if using a heterologous host. The authors state that there are >1500 similar gene clusters suitable for this approach, a number which is certain to increase exponentially as more microbial genomes are sequenced (it is conservatively estimated that >1 million bacterial and fungal genomes will have been sequenced over the next 10 years) [15], particularly from novel or under-represented phyla. Using a similar approach, two antimicrobials were identified and synthesized; one with antibacterial and one with antifungal activity following a screen against ESKAPE (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) pathogens and *Candida albicans* [17].

The potential to identify novel BGCs and products thereof is enormous, especially when one considers that an estimated 99% of microbes are as yet uncultured [18]. Even among the best-studied natural product producers, the Streptomycetes, it is estimated that only 5% of their predicted 150,000 natural products have been characterized to date [19,20]. Improved bioinformatic algorithms, advances in long-read sequencing (e.g., PacBio and Oxford Nanopore) and single-celled genomics, coupled with decreases in the cost of DNA sequencing and synthesis will provide a means to access the enormous diversity of BGCs and develop novel antimicrobials. This will also bypass the need to culture the producing organism or heterologously express BGCs by synthesizing the compound directly. In addition to this, synthetic biology is likely to play a key role in the design, modification and production of natural products from BCGs [19]. Modifying or creating new genetic circuits to improve gene expression and to introduce strong promoters and regulators may be helpful to produce larger, more intricate compounds, which currently are too complex for direct synthesis.

Against this backdrop, a number of collaborative projects, as part of the National Microbiome Initiative, are investing heavily to identify novel antimicrobials as part of natural product discovery pipelines [21]. Additionally, the Bill and Melinda Gates Foundation has provided \$17 million USD start-up funding

“The potential to identify novel biosynthetic gene clusters and products thereof is enormous...”

for Lodo Therapeutics in a similar vein [22]. Such continued investment is vital to drive this area of research forward, to identify, characterize and produce novel antimicrobials and ultimately to translate such discoveries to viable clinical therapeutics effective against emerging multidrug-resistant pathogens.

In the words of Winston Churchill, in the context of a different global conflict, “This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning.”

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